REMARKS

This Preliminary Amendment is filed as part of a Continuing Prosecution Application (CPA).

In an Office Action mailed November 7, 2001, the newly appointed Examiner in charge of this application made final a requirement for restriction to Group I, Claims 1-9 and Claims 26-29. The Examiner withdrew rejections of Claims 1, 3-6, 8, 9 and 26 for obviousness-type double patenting over Claims 1-3 and 6 of US Patent No. 5,780,236. The Examiner agreed that Claims 26-29 are fully enabled by the specification, but maintained rejections of Claims 1-9 for overbreadth. The Examiner maintained rejections under 35 U.S.C. §102(a) of certain claims, including claims that are withdrawn from consideration, as being anticipated by Bilger et al. It is not entirely clear from the Examiner's statements on pages 7 and 8 which claims are rejected for anticipation by Bilger et al. Reference is made to Claims 1-5 and 26-29 as well as to Claims 6, 8, and 9. Finally, the Examiner maintained rejections of Claims 2 and 27 over Bilger et al. in combination with Rinchik et al. and Dietrich et al., respectively for obviousness under 35 U.S.C. §103(a).

A request for extension of time in the underlying case for two months accompanies this Preliminary Amendment, so that the CPA will be deemed to have been timely filed. Should any other extension of time be due in this or any subsequent response, please consider this to be a request for the appropriate extension of time and a request to charge the fee due to Deposit Account No. 17-0055.

Other than the CPA filing fee and the extension of time fee, no other fee is believed due in connection with this filing. However, should any additional fee be due, please charge the fee to the same deposit account.

In the prior response by Applicants, Applicants indicated that a petition for correction of inventorship would be filed. The Petition accompanies the CPA. Favorable action on the Petition is respectfully requested.

Each issued raised by the Examiner is considered separately below. Reconsideration is respectfully requested.

Requirement for Restriction

Claims 10-25 stand withdrawn from further consideration notwithstanding the applicants' request for reconsideration of the restriction requirement. Applicants note that the Examiner failed to complete a sentence commenting on the applicants' arguments. Absent the Examiner's reason for making final the restriction requirement, Applicants cannot respond. To the extent such comment was intended, applicants respectfully ask that the comments be included in a subsequent office communication.

Rejections Under 35 U.S.C. §112, first paragraph

The Examiner agreed that Claims 26-29, limited to use of isogenic mouse strains, are free of this rejection. However, Claims 1-9 stand rejected for overbreadth. Specifically, the Examiner believes that the applicants present no nexus between using the claimed methods in highly defined inbred mice strains and extending said methods for use in other non-human mammals. Applicants respectfully traverse the rejection, but, in the interest of moving prosecution forward, also include new Claims 30-42 which specifically recite in each case that the animals used are mice.

The basis for the applicants' traversal is as follows. The specification notes at page 3, lines 31-32 that inbred strains of mice, rats, and rabbits exist. The skilled artisan is familiar with those strains and with the genetics and physiology of those animals. Any gene identified in such animals that can be characterized by a phenotype is suitably used in the methods of the claimed invention. For the reasons discussed in prior responses, the methods of the invention are applicable to any animal system having Mendelian inheritance. The skilled artisan working with any of those species can readily select a dominant index allele as has been done in the mouse case by the applicants, and can study modifications of such alleles in precisely the manner disclosed in this patent application. If the mere absence from the record of the identity of such inbred strains is the sole basis for refusing to extend the scope of the claims to non-mouse animals, applicants believe that the existence of such strains is readily established by the *Index of Inbred Strains of Mice and Rats* maintained by Michael FW Festing, MRC Toxicology Unit, Hodgkin Building, University of Leicester, UK on the website of The Jackson Laboratory, Bar Harbor, Maine (www.jax.org). The website refers to numerous prior publications concerning inbred rat strains, dating back to 1971. Further, texts

such as *Inbred and Genetically Defined Strains of Laboratory Animals,* Altman and Katz (eds.) (FASEB 1979) are available. Part 1 of the aforementioned book describes mouse and rat strains, while Part 2 describes hamster, guinea pig, rabbit and chicken strains. Unfortunately, a copy of the book is not readily available to the Applicants.

The introduction of a transgene to confer a dominant index phenotype into the inbred strains is an art-recognized technique that has been applied, e.g., in the outbred Sprague Dawley rat by Hully, J. R. et al., "Transgenic Hepatocarcinogenesis in the Rat, Am. J. Pathol. 145:481 (1994). In Hully et al., an outbred rat strain was used in a study of transgenic hepatocarcinogenesis, where the hepatocarcinogenesis phenotype acts as an index phenotype. Had the mice been inbred, the same priniciples would apply, with the additional advantage of background homozygosity. No additional insight or experimentation is required to extend the methods of Hully to inbred rats. The very same mutagenic steps applied to the founder inbred mouse by the Applicants can be applied in an underlying inbred rat to produce a founder animal having point mutations. When crossed to the index inbred rat, offspring having an altered phenotype (e.g., hepatocarcinogenic profile such as susceptibility, severity or the like) can be obtained and analyzed as disclosed. One can consider the outbred transgenic rats of the Hully paper as analogous starting materials to the inbred B6-Min mice of the Applicant's Example, in that both have a modifiable phenotype, keeping in mind that the points of difference are no impediment to the skilled artisan in possession of the invention.

For all these reasons, it is believed that the Examiner has not met his burden of showing a lack of enablement. Rather, the Applicants have presented evidence that the invention can be practiced without undue experimentation by a skilled artisan using available tools, particularly inbred animal strains other than mice. Reconsideration and withdrawal of these rejections are respectfully requested.

Rejections Under 35 U.S.C. §102(a)

The Examiner maintained rejections for anticipation by Bilger et al. under 35 U.S.C. §102(a). It is not clear whether the rejected claims are Claims 1 and 3-25 or Claims 1-5 and 26-29, as both are mentioned in the rejection, as are Claims 6, 7 and 8. In any event, applicants believe that the Examiner's rejection is flawed. The Examiner has apparently

picked and chosen pieces of Bilger et al. to formulate a rejection, but in doing so misstates the founder inbred strain requirements, mischaracterizes the "mutation" status in AKR mice, misinterprets the non-isogenic nature Bilger's B6.*Mom1*^{akr} animals, and misunderstands Bilger's reference to using ENU to produce B6-Min mice, as follows:

1. The Examiner asserts that the only requirement of the founder strain is that it contain a single point mutation. Rather, the strain must contain random point mutations relative to a wild-type animal of the founder inbred strain.

2. As an example of such a mutation in the founder, the Examiner states that Bilger showed a frame shift mutation in AKR that results in a truncated protein. In fact, AKR does not contain a frame shift mutation. The paper states that AKR and five other strains having tumor resistance alleles of *Mom1* encode full length Pla2g2a. Three different strains that carry a susceptibility allele at *Mom1* include the frameshift mutation.

3. With regard to Claim 6 (wherein the founder is isogenic with the index inbred strain), the Examiner looks to the backcross of the AKR allele into B6 in Bilger. This is improper for two reasons. First, the AKR allele was taken from a wild-type AKR mouse and contains no differences from the wild-type AKR animal. Second, the AKR allele introduced into the B6 background through multiple generations of backcrossing did not create an animal isogenic with the B6 index inbred strain. Rather, B6.*Mom1*^{akr} animals carrying the AKR *Mom1* region contained a substantial 35 cM segment of AKR DNA.

4. The Examiner argues that Bilger et al. described using ENU to define the Min mutant in phenotypic screens. While true, this is not how a mutagenic agent is used in pending Claims 8 and 9 where the agent induces point mutations in a wild-type founder inbred mouse to produce founder animals. The Examiner confused the founder animals with the index inbred strain carrying a congenic dominant allele at a locus known to confer the index phenotype.

At its core, the paper by Bilger et al. describes a search for polymorphic modifiers arising from strain differences among various inbred mouse strains (e.g., AKR and C57BL/6). In such discovery efforts, one cannot point to a single mutation as the cause of an observed change. In the crosses of prior methods, including Bilger, polymorphic strain

differences introduce a plurality of changes that can together yield an observed difference. In the present gene discovery method, by contrast, a single change modifies the index phenotype. The single change can be mapped and identified as disclosed by the Applicants. Such specificity was not available to or contemplated by Bilger et al. For all of the aforementioned reasons, the stated grounds for rejection under §102(a) over Bilger et al. cannot stand. Reconsideration and withdrawal of these rejections are respectfully requested.

Rejections Under 35 U.S.C. §103(a)

Claims 2 and 27 remain rejected over Bilger et al. in view of Rinchik et al. and Dietrich et al., respectively. As we have argued above, applicants believe that these combination rejections cannot stand because of the flaws in the underlying rejection over Bilger et al., particularly in view of the deficiencies in the rejection under §102(a) described above. Reconsideration and withdrawal of these rejections are respectfully requested.

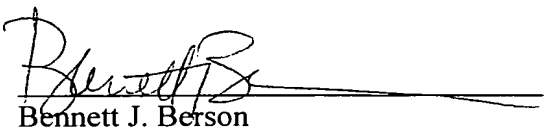
Amended Claims 27 and 28

A reference in Claim 27 to "mapping LOD scores" was not accurate. The Claim is amended to more accurately refer to "ranking the LOD scores". Support for the amendment is found in the specification on page 16, line 25.

Claim 28 is amended to clarify that the mapping partner strain has the genetic background of the index inbred strain. Claim 6, upon which Claim 28 depends, already recites that the index inbred strain and the founder inbred strain share an isogenic genetic background. Support for the amendment to Claim 28 is found in the specification on page 15, line 7-10.

Reconsideration of the merits of this patent application is respectfully requested.

Respectfully submitted,



Bennett J. Berson
Reg. No. 37,094
Attorney for Applicants
QUARLES & BRADY LLP
P.O. Box 2113
Madison, WI 53701-2113

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Applicants: William F. Dove
Alexandra Shedlovsky

Date: April 8, 2002

Serial No.: 09/114,973

Group Art Unit: 1632

Filed: 07/14/98

Examiner: J. Woitach

Title: METHOD FOR IDENTIFYING MUTANTS AND MOLECULES

Docket No.: 960296.95491

1. A method for identifying a segregating single point mutation at a genetic locus that modifies an index phenotype in a non-human index inbred strain, the segregating mutation causing an outlying phenotype relative to the index phenotype, the method comprising the steps of:

outcrossing at least one male animal of a non-human founder inbred strain to at least one female animal of a non-human index inbred strain to obtain F1 progeny, the founder inbred strain carrying random point mutations relative to a wild-type animal of the founder inbred strain, the index inbred strain carrying a congenic dominant allele at a locus known to confer the index phenotype and being genetically distinguishable from the founder inbred strain, wherein at least one of the F1 progeny that carry the dominant allele also carry at least one random mutation;

backcrossing gametes from male F1 progeny to at least one female of the index inbred strain, with or without the index allele, to obtain N2 backcross progeny, wherein at least one of the N2 backcross progeny that carry the dominant allele also exhibit the outlying phenotype; and

verifying that the outlying phenotype is caused by a segregating single point mutation.

2. A method as claimed in Claim 1 wherein any of the crosses employ preserved gametes.

3. A method as claimed in Claim 1 wherein the F_1 progeny and some of the N2 progeny exhibit an extreme outlying phenotype.

4. A method as claimed in Claim 3 wherein the segregating mutation is a heterozygous modifier of the index phenotype selected from a group consisting of an enhancing modifier and a suppressing modifier.

5. A method as claimed in Claim 1 wherein the dominant allele is a *Min* allele at an *Apc* locus in a mouse.

6. A method as claimed in Claim 1 wherein the index inbred strain and the founder inbred strain share an isogenic genetic background.

7. A method as claimed in Claim 6 further comprising the step of mapping the segregating mutation using a mapping partner strain produced by the steps of:

treating an animal of an index strain with a mutagenic agent to induce point mutations in the treated animal;

crossing the treated animal to an animal of the index strain to produce F1 progeny; and

sib-mating F1 and subsequent generation progeny until detrimental and lethal mutations are eliminated.

8. A method as claimed in Claim 1 wherein the founder inbred mouse strain is produced by a method comprising the step of treating a wild-type inbred mouse with a mutagenic agent to induce point mutations.

9. A method as claimed in Claim 8 wherein the mutagenic agent is ethylnitrosourea.

26. A method as claimed in Claim 6 wherein the method identifies a segregating mutation at a genetic locus that modifies tumor multiplicity in a C57BL/6 mouse congenic for the *Min* allele at the Apc locus, the method comprising the steps of:

outcrossing at least one male C57BL/6 mouse carrying random point mutations to a female C57BL/6 mouse congenic for the *Min* allele at the Apc locus to obtain F1 progeny, wherein at least one of the F1 progeny carries both the *Min* allele and a random point

mutation; and

backcrossing gametes from male F1 progeny to at least one female C57BL/6 mouse congenic for the *Min* allele at the Apc locus to obtain N2 backcross progeny, wherein at least one of the N2 backcross progeny carries the *Min* allele and has a tumor multiplicity that is modified relative to tumor multiplicity in a C57BL/6 mouse congenic for the *Min* allele at the Apc locus, the modified tumor multiplicity being characteristic of the segregating mutation.

27. (Amended) A method as claimed in Claim 26 wherein the modified tumor multiplicity is evaluated according to a method comprising the steps of:

repeatedly applying for random permutations of mice among N2 backcross subkindreds a likelihood ratio test of the null hypothesis that no multiplicity modifier is segregating to obtain a p-value, wherein a p-value of less than 0.05 indicates a potential carrier of the segregating mutation;

when the p-value is less than 0.05, calculating, for each potential carrier that has offspring with information about tumor multiplicity, a LOD score for presence of the segregating mutation, wherein the LOD score is $\log_{10}$ of a ratio of the probability of offspring phenotype data if the potential carrier mouse carries a multiplicity modifier to the probability of offspring phenotype data if the potential carrier mouse does not carry a multiplicity modifier, and wherein the denominator probabilities are calculated from an estimated background distribution and the numerator probabilities are calculated from a mixture of the estimated background distribution and an estimated modified distribution, where the estimated distributions are obtained by the method of maximum likelihood; and

[mapping] <u>ranking the</u> LOD scores of the potential carriers, whereby animals having the highest LOD scores are likely carriers of the segregating mutation.

28. (Amended) A method as claimed in claim 26, further comprising the step of mapping the segregating mutation in the N2 backcross progeny using a mapping partner strain <u>having the genetic background of the index inbred strain</u>.

29. A method as claimed in Claim 28 wherein the mapping partner strain is produced by the steps of:

treating a C57BL/6 mouse with a mutagen to introduce random point mutations;

crossing the treated mouse to a C57BL/6 mouse to produce F1 progeny; and

sib-mating F1 and subsequent generation progeny until detrimental and lethal mutations are eliminated.

30. (New) A method for identifying a segregating single point mutation at a genetic locus that modifies an index phenotype in a mouse index inbred strain, the segregating mutation causing an outlying phenotype relative to the index phenotype, the method comprising the steps of:

outcrossing at least one male animal of a mouse founder inbred strain to at least one female animal of a mouse index inbred strain to obtain F1 progeny, the founder inbred strain carrying random point mutations relative to a wild-type animal of the founder inbred strain, the index inbred strain carrying a congenic dominant allele at a locus known to confer the index phenotype and being genetically distinguishable from the founder inbred strain, wherein at least one of the F1 progeny that carry the dominant allele also carry at least one random mutation;

backcrossing gametes from male F1 progeny to at least one female of the index inbred strain, with or without the index allele, to obtain N2 backcross progeny, wherein at least one of the N2 backcross progeny that carry the dominant allele also exhibit the outlying phenotype; and

verifying that the outlying phenotype is caused by a segregating single point mutation.

31. (New) A method as claimed in Claim 30 wherein any of the crosses employ preserved gametes.

32. (New) A method as claimed in Claim 30 wherein the F_1 progeny and some of the N2 progeny exhibit an extreme outlying phenotype.

33. (New) A method as claimed in Claim 32 wherein the segregating mutation is a heterozygous modifier of the index phenotype selected from a group consisting of an enhancing modifier and a suppressing modifier.

34. (New) A method as claimed in Claim 30 wherein the dominant allele is a *Min* allele at an *Apc* locus in a mouse.

35. (New) A method as claimed in Claim 30 wherein the index inbred strain and the founder inbred strain share an isogenic genetic background.

36. (New) A method as claimed in Claim 35 further comprising the step of mapping the segregating mutation using a mapping partner strain produced by the steps of:

treating an animal of an index strain with a mutagenic agent to induce point mutations in the treated animal;

crossing the treated animal to an animal of the index strain to produce F1 progeny; and

sib-mating F1 and subsequent generation progeny until detrimental and lethal mutations are eliminated.

37. (New) A method as claimed in Claim 30 wherein the founder inbred mouse strain is produced by a method comprising the step of treating a wild-type inbred mouse with a mutagenic agent to induce point mutations.

38. (New) A method as claimed in Claim 37 wherein the mutagenic agent is ethylnitrosourea.

39. (New) A method as claimed in Claim 35 wherein the method identifies a segregating mutation at a genetic locus that modifies tumor multiplicity in a C57BL/6 mouse congenic for the *Min* allele at the Apc locus, the method comprising the steps of:

outcrossing at least one male C57BL/6 mouse carrying random point mutations to a female C57BL/6 mouse congenic for the *Min* allele at the Apc locus to obtain F1 progeny, wherein at least one of the F1 progeny carries both the *Min* allele and a random point mutation; and

backcrossing gametes from male F1 progeny to at least one female C57BL/6 mouse congenic for the *Min* allele at the Apc locus to obtain N2 backcross progeny, wherein at least one of the N2 backcross progeny carries the *Min* allele and has a tumor multiplicity that is

modified relative to tumor multiplicity in a C57BL/6 mouse congenic for the *Min* allele at the Apc locus, the modified tumor multiplicity being characteristic of the segregating mutation.

40. (New) A method as claimed in Claim 39 wherein the modified tumor multiplicity is evaluated according to a method comprising the steps of:

repeatedly applying for random permutations of mice among N2 backcross subkindreds a likelihood ratio test of the null hypothesis that no multiplicity modifier is segregating to obtain a p-value, wherein a p-value of less than 0.05 indicates a potential carrier of the segregating mutation;

when the p-value is less than 0.05, calculating, for each potential carrier that has offspring with information about tumor multiplicity, a LOD score for presence of the segregating mutation, wherein the LOD score is $\log_{10}$ of a ratio of the probability of offspring phenotype data if the potential carrier mouse carries a multiplicity modifier to the probability of offspring phenotype data if the potential carrier mouse does not carry a multiplicity modifier, and wherein the denominator probabilities are calculated from an estimated background distribution and the numerator probabilities are calculated from a mixture of the estimated background distribution and an estimated modified distribution, where the estimated distributions are obtained by the method of maximum likelihood; and

ranking the LOD scores of the potential carriers, whereby animals having the highest LOD scores are likely carriers of the segregating mutation.

41. (New) A method as claimed in claim 39, further comprising the step of mapping the segregating mutation in the N2 backcross progeny using a mapping partner strain.

42. (New) A method as claimed in Claim 41 wherein the mapping partner strain is produced by the steps of:

treating a C57BL/6 mouse with a mutagen to introduce random point mutations;

crossing the treated mouse to a C57BL/6 mouse to produce F1 progeny; and

sib-mating F1 and subsequent generation progeny until detrimental and lethal mutations are eliminated.